

AMENDMENTS TO THE CLAIMS:

Amend the claims as follows.

Claims 1-106. (Canceled)

107. (Previously Presented) A method for producing a luciferase which is substantially free of enzymatically active *E.coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase which is thermostable at 37°C, and expresses adenylate kinase only in a mutant form which form is denatured at a temperature of 37°C; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

108. (Currently Amended) A method according to claim 107 wherein the luciferase is a luciferase selected from the group consisting of ~~*Photinus pyralis*~~*Photinus pyralis* luciferase which has a mutation at position 354 in the amino acid sequence, and a ~~*Luciola*~~*Luciola* luciferase with a mutation at position ~~[[354]]~~356, which mutation elevates the thermostability of the protein over that of the wild-type protein.

109. (Currently Amended) A method according to claim 107 wherein the luciferase is a ~~Luciola~~Luciola luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid.

110. (Previously Presented) A method according to claim 107 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

111. (Previously Presented) A method according to claim 107 wherein the adenylate kinase comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase.

112. (Previously Presented) A method according to claim 107 wherein the said temperature is a temperature of from 37°C up to a temperature below which the luciferase is denatured.

113. (Previously Presented) A recombinant *E. coli* cell which has been transformed so that it expresses a first nucleotide sequence which encodes a luciferase which is stable at 37°C under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which is denatured at 37°C.

114. (Previously Presented) A recombinant cell according to claim 113 which further comprises at least one selection marker.

115. (Currently Amended) A recombinant cell according to claim 113 wherein the luciferase is a ~~Photinus pyralis~~Photinus pyralis luciferase which has a mutation at position 354 in the amino acid sequence, or a ~~Luciola~~Luciola luciferase with a mutation at position ~~[[354]]~~356, which mutation elevates the thermostability of the protein over that of the wild-type protein.

116. (Currently Amended) A recombinant cell according to claim 113 wherein the luciferase is a ~~Luciola~~Luciola luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid.

117. (Currently Amended) A method for producing a recombinant cell according to claim 113 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which is denatured at 37°C, subjecting transformants to a temperature of 37°C or more and detecting those which do not grow as a result of lack of active ~~[[anenylate]]~~adenylate kinase, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

118. (Previously Presented) A method according to claim 117 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

119. (Previously Presented) A method according to claim 118 wherein said selection markers comprise different antibiotic resistance genes.

120. (Currently Amended) A method for producing a luciferase which is substantially free of enzymatically active *E.coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase selected from the group consisting of ~~*Photinus pyralis*~~*Photinus pyralis* luciferase which has a mutation at position 354 in the amino acid sequence, and a ~~*Luciola*~~*Luciola* luciferase with a mutation at position ~~[[354]]~~356, which mutation elevates the thermostability of the protein over that of the wild-type protein, and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

121. (Previously Presented) A method according to claim 120 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the

luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

122. (Previously Presented) A method according to claim 121 wherein the said temperature is a temperature of from 37°C up to a temperature below which the luciferase is denatured.

123. (Currently Amended) A recombinant *E. coli* cell which has been transformed so that it expresses a luciferase selected from the group consisting of ~~*Photinus pyralis*~~*Photinus pyralis* luciferase which has a mutation at position 354 in the amino acid sequence, and a ~~*Luciola*~~*Luciola* luciferase with a mutation at position ~~[[354]]~~356, which mutation elevates the thermostability of the protein over that of the wild-type protein, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase.

124. (Previously Presented) A recombinant cell according to claim 123 which further comprises at least one selection marker.

125. (Currently Amended) A method for producing a recombinant cell according to claim 123 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which comprises a mutation at amino

acid 87 or 107 in the sequence of *E. coli* adenylate kinase, subjecting transformants to a temperature of 37°C or more and detecting those which do not grow as a result of lack of active ~~[[anenylate]]~~adenylate kinase, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

126. (Previously Presented) A method according to claim 125 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

127. (Currently Amended) A method according to claim 126 wherein said selection markers comprise ~~particular~~ different antibiotic resistance genes.

128. (Currently Amended) A method for producing a luciferase which is substantially free of enzymatically active *E. coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a ~~Luciola~~Luciola luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid, and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

129. (Previously Presented) A method according to claim 128 wherein the *E. coli* host cells are cultured for a period which is sufficient to allow production of the luciferase, and then a batch of said culture is subjected to a temperature at which the adenylate kinase is denatured, and the luciferase is recovered from the said batch.

130. (Previously Presented) A method according to claim 128 wherein the said temperature is a temperature of from 37°C up to a temperature below which the luciferase is denatured.

131. (Currently Amended) A recombinant *E. coli* cell which has been transformed so that it expresses a ~~Luciola~~Luciola luciferase in which the amino acid at the 217 position is mutated to a hydrophobic amino acid, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase.

132. (Previously Presented) A recombinant cell according to claim 131 which further comprises at least one selection marker.

133. (Previously Presented) A method for producing a recombinant cell according to claim 131 which method comprises in any order (a) transforming a host cell

with a vector which encodes adenylate kinase in a form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase, subjecting transformants to a temperature of 37°C or more and detecting those which do not grow as a result of lack of active adenylate kinase, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

134. (Previously Presented) A method according to claim 133 wherein the vector which encodes said adenylate kinase further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

135. (Previously Presented) A method according to claim 134 wherein said selection markers comprise different antibiotic resistance genes.

136. (new) A method according to claim 107 wherein the luciferase is a luciferase selected from the group consisting of *Photinus pyralis* luciferase which has a mutation at amino acid position 354 in the amino acid sequence with reference to the wild-type *Photinus pyralis* luciferase sequence (SEQ ID NO: 1), and a *Luciola* luciferase with a mutation at amino acid position 356 with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4), which mutation elevates the thermostability of the protein over that of the wild-type protein.

137. (new) A method according to claim 107 wherein the luciferase is a *Luciola* luciferase in which the amino acid at the 217 position with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4) is mutated to a hydrophobic amino acid.

138. (new) A method according to claim 107 wherein the adenylate kinase includes mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5).

139. (new) A recombinant cell according to claim 113 wherein the luciferase is a *Photinus pyralis* luciferase which has a mutation at amino acid position 354 with reference to the wild-type *Photinus pyralis* luciferase sequence (SEQ ID NO: 1) in the amino acid sequence, or a *Luciola* luciferase with a mutation at amino acid position 356 with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4), which mutation elevates the thermostability of the protein over that of the wild-type protein.

140. (new) A recombinant cell according to claim 113 wherein the luciferase is a *Luciola* luciferase in which the amino acid at the 217 position with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata*

luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4) is mutated to a hydrophobic amino acid.

141. (new) A method of claim 120, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a luciferase selected from the group consisting of *Photinus pyralis* luciferase which has a mutation at amino acid position 354 with reference to the wild-type *Photinus pyralis* luciferase sequence (SEQ ID NO: 1) in the amino acid sequence, or a *Luciola* luciferase with a mutation at amino acid position 356 with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4), which mutation elevates the thermostability of the protein over that of the wild-type protein, and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5); and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

142. (new) A recombinant *E. coli* cell of claim 123 which has been transformed so that it expresses a luciferase selected from the group consisting of *Photinus pyralis* luciferase which has a mutation at amino acid position 354 with reference to the wild-type *Photinus pyralis* luciferase sequence (SEQ ID NO: 1) in the amino acid sequence,

and a *Luciola* luciferase with a mutation at position 356 with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4), which mutation elevates the thermostability of the protein over that of the wild-type protein, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5).

143. (new) A method for producing a recombinant cell according to claim 123 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5), subjecting transformants to a temperature of from 37°C or more and detecting those which do not grow as a result of lack of active anenylate adenylate kinase, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.

144. (new) A method of claim 128 for producing a luciferase which is substantially free of enzymaticall active *E.coli* adenylate kinase, the method comprising culturing an *E. coli* host cell which has been transformed so that it expresses a *Luciola*

luciferase in which the amino acid at the 217 position with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4) is mutated to a hydrophobic amino acid, and expresses adenylate kinase and expresses adenylate kinase only in a mutant form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5); and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to a temperature at which the adenylate kinase is denatured but the luciferase remains substantially unaffected, so as to denature the adenylate kinase.

145. (new) A recombinant *E. coli* cell of claim 131 which has been transformed so that it expresses a *Luciola* luciferase in which the amino acid at the 217 position with reference to the wild-type *Luciola* luciferase sequences selected from the group consisting of *L. cruciata* luciferase (SEQ ID NO: 2), *L. lateralis* luciferase (SEQ ID NO: 3) and *L. mingrelica* luciferase (SEQ ID NO: 4) is mutated to a hydrophobic amino acid, under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses adenylate kinase only in a mutated form and expresses adenylate kinase only in a mutant form which has mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5).

146. (new) A method of claim 133 for producing a recombinant cell according to claim 131 which method comprises in any order (a) transforming a host cell with a vector which encodes adenylate kinase in a form which comprises a mutation at amino acid 87 or 107 in the sequence of *E. coli* adenylate kinase with reference to the wild-type sequence of *E. coli* adenylate kinase (SEQ ID NO: 5), subjecting transformants to a temperature of 37°C or more and detecting those which do not grow as a result of lack of active adenylate kinase, and (b) transforming said host cell with a vector which encodes the said luciferase and a first selection marker, and using the first selection marker to detect stable transformants.